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E. B. KALAN

Eastern Marketing and Nutrition Research Division, Agricultural Research Service
U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118, U.S.A.

R. R. KRAELING, AND R. J. GERRITS

Animal Husbandry Research Division, Agricultural Research Service,
U.S. Department of Agriculture, Beltsville, Maryland 20705, U.S.A.

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R. R. KRAELING,† AND R. J. GERRITS

Animal Husbandry Research Division, Agricultural Research Service,
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ABSTRACT

1. Two polymorphic forms of a swine whey protein have been isolated, purified, and crystallized.

2. The polymorphs show differences of electrophoretic mobility in alkaline gels despite the finding that the only compositional difference is in alanine and valine content.

3. The proteins have a molecular weight of ~18,000, isoionic point of 4·78–4·80, no sulphydryls, and *C*- and *N*-terminal valine.

4. A case can be made that the proteins are homologous to ruminant β-lactoglobulin but not to α-lactalbumin.

THE occurrence of genetic polymorphism in the bovine whey protein, β-lactoglobulin, was first reported by Aschaffenburg and Drewry (1955, 1957a). Filter paper electrophoresis with a veronal buffer, *p*H 8·6, resolved the β-lactoglobulin into 2 components, the faster designated A, the slower B. Genetic data of Aschaffenburg and Drewry (1955, 1957a), Plowman, Townend, Kiddy, and Timasheff (1959), Groves, Peterson, and Kiddy (1965) demonstrated that the β-lactoglobulin polymorphs are controlled by 2 codominant alleles producing the phenotypes β-lactoglobulin A, β-lactoglobulin AB, and β-lactoglobulin B. A third polymorph, β-lactoglobulin C, was discovered by Bell (1962) in Australian Jersey cows and a fourth, β-lactoglobulin D, by Grosclaude, Pujolle, Garnier, and Ribadeau-Dumas (1966) in the Montbeliarde breed. Bell and McKenzie (1964) and Maubois, Pion, and Ribadeau-Dumas (1965) reported the existence of polymorphic β-lactoglobulins of ewe's milk and a suspected polymorphism of β-lactoglobulin of doe's milk has been reported by Lyster, Jenness, Phillips, and Sloan (1966). Multiple forms of α-lactalbumin of cow's milk were first described by Blumberg and Tombs (1968) and later by Bhattacharya, Roychoudhury, Sinha, and Sen (1963). The discovery of other polymorphic whey proteins is anticipated.

The present report describes the isolation and partial characterization of 2 variants of a polymorphic protein from sow's whey. The protein has an amino-acid composition which is, overall, similar to that of cow β-lactoglobulin, but its solubility characteristics are more akin to cow α-lactalbumin. It is interesting that the 2 polymorphs differ only in alanine and valine content, yet they show differences in electrophoretic mobility in veronal buffer, *p*H 8·6, in 5 per cent acrylamide gel.

Preliminary reports by Kalan, Kraeling, and Gerrits (1967, 1968) have been published, and more recently Kraeling and Gerrits

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† Present address: Department of Animal Science, Iowa State University, Ames, Iowa 50010.

(1969) have reported on the genetics of the polymorphism and distribution of the proteins in 3 porcine breeds. Most recently Kessler and Brew (1970) have also reported on a whey protein from pig's milk which appears to be similar, if not identical, to the polymorph designated AA in the present paper.

MATERIALS AND METHODS

Isolation of Proteins

Each of the polymorphic proteins was isolated in identical fashion with similar yield. A description of the isolation of the polymorphic protein designated BB, based on its slower mobility in gel electrophoresis, is given below.

Raw milk, 5·4 litres, was heated to 40° C., and the cream was removed by passing the milk twice through a DeLaval separator.* The skim milk, *p*H 7·1, was then adjusted to *p*H 4·0 with 460 ml. of 1·0 *N* HCl with stirring, and the casein removed by centrifugation. In larger scale preparations, the casein was more conveniently separated by filtration through cheesecloth sacks reinforced by an outer felt-cloth bag. The turbid filtrate, 3·25 litres, was adjusted to *p*H 6·7 with 173 ml. of 1·0 *N* NaOH and 20 g. of Na_2SO_4 were added with stirring for each 100 ml. of solution. The precipitate was removed by filtration and the *p*H of the filtrate was adjusted by careful addition of 21–22 ml. of 6 *N* HCl. A persistent turbidity developed at *p*H 5·1–5·0 which increased as the *p*H was lowered to 3·0. The precipitate was removed by filtration, scraped from the filter paper into dialysis tubing, and dialysed under toluene against running tap-water. The filtrate, 2·7 litres, was adjusted to *p*H 6·0 with 7·5 *N* NH_4OH; 540 g. $(NH_4)_2SO_4$ were added with stirring. A faint turbidity, which did not increase upon standing overnight, developed and this fraction, which ordinarily contains β-lactoglobulin when cow's milk is treated in this fashion, was discarded.

The precipitate obtained at *p*H 3·0 in the presence of 20 per cent Na_2SO_4 was almost completely soluble after dialysis. The dialysate, which was filtered and lyophilized, yielded 21 g. of solids of which 5·0 g. were subjected to a *p*H fractionation in the presence of 20 per cent Na_2SO_4.

Five g. of solids were dissolved in 200 ml. H_2O resulting in a solution of *p*H 6·5. Na_2SO_4, 40 g., was added with stirring, and after solution, fractions were taken at *p*H 5, *p*H 4, and *p*H 3 after addition of 1·0 *N* HCl. Each fraction was dialysed and then lyophilized. The fraction obtained at *p*H 4, amounting to 3·40 g., was the fraction of interest and was subjected to an ammonium sulphate fractionation at *p*H 6·0.

Three g. of the *p*H 4·0, 20 per cent Na_2SO_4 fraction were dissolved in 100 ml. H_2O and the solution was adjusted to *p*H 6·0 with 1·0 *N* NH_4OH. Fractions were taken at 2·3 *M* and 3·3 *M* $(NH_4)_2SO_4$ after the addition of the solid salt with stirring. Each fraction was dialysed and lyophilized. Protein, 1·85 g., was obtained from the 2·3 *M* $(NH_4)_2SO_4$ fraction which was treated as follows.

One g. of the 2·3 *M* $(NH_4)_2SO_4$ fraction was dissolved in 50 ml. H_2O, and the solution was adjusted to *p*H 6·2. Fifty ml. of a saturated $(NH_4)_2SO_4$ solution, *p*H 6·7, were added and the turbidity formed was removed by centrifugation. The supernatant was brought to 55 per cent saturation by the dropwise addition of 10 ml. of saturated $(NH_4)_2SO_4$. The precipitate which resulted was collected by centrifugation, transferred to dialysis tubing, and dialysed free of salt and lyophilized; 610 mg. of protein were obtained. When 300 mg. of this material were again treated with saturated $(NH_4)_2SO_4$, as described above, a birefringence developed at 55 per cent saturation and small, needle-like crystals were observed under the microscope. The crystals were removed by centrifugation, dissolved in H_2O, dialysed and lyophilized. A second crop was obtained by addition of saturated $(NH_4)_2SO_4$ to the supernatant until turbidity developed. A total of 190 mg. of crystalline material was obtained. This represents a yield of 16·2 per cent based on the *p*H 3, 20 per cent Na_2SO_4 precipitate of 21 g. A flow chart of the preparation is shown in *Fig.* 1.

Polyacrylamide Gel Electrophoresis

The conditions used were essentially those of Groves and others (1965). A 2·0-mg. sample was dissolved in 1·0 ml. buffer and 200 mg. of sucrose were added. A solution of 20 µl. was used in a 5 per cent polyacrylamide gel, *p*H 8·6, veronal buffer, 0·025 ionic strength. Each run was for $3\frac{1}{2}$ hours at 60 mA. (about 200 V.) and 5° C. The proteins were visualized with Amido black.

Amino-acid Analysis

Samples of 1·8–2·0 mg. of the once recrystallized proteins were hydrolysed at 110° C. in duplicate for 24, 48, and 100 hours with 1·0 ml. of redistilled 5·7 *N* HCl in sealed, evacuated tubes. The hydrolysates were stored at −20° C. The amino-acids were separated and quantitated using an automatic amino-acid analyser according to the method of Piez and Morris (1960). Residue numbers were calculated from molar ratios based on 12 different amino-acids (Asp, Glu, Pro, Gly, Ala, Val, Ile, Leu, Phe, Lys, His, and Arg) which showed no increase or decrease over the period of hydrolysis. The values based on each of the 12 amino-acids were then averaged for each of the 6

* It is not implied that the U.S. Department of Agriculture recommends the above company or its products to the exclusion of any others.

determinations and finally a grand average was obtained for each protein and is reported in *Table I*. Threonine, serine, and methionine were obtained from the data by the least squares method of linear regression analysis. Cystine apparently suffered destruction of about 10–15 per cent and

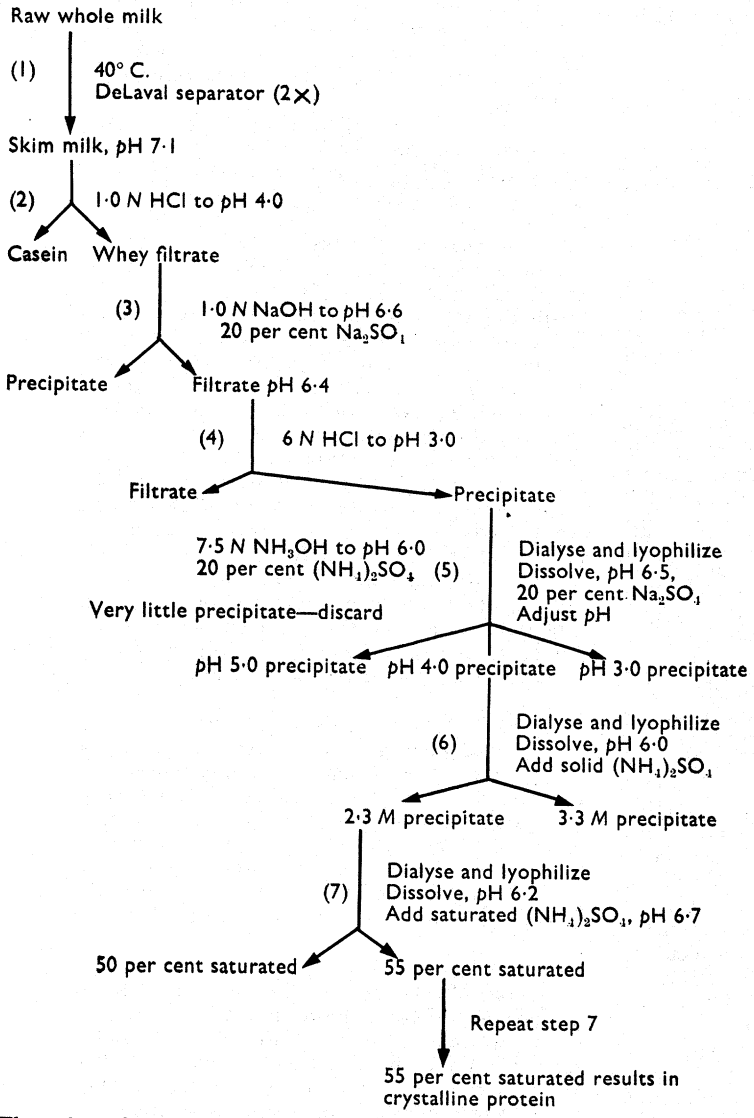

FIG. 1.—Flow chart for preparation of crystalline polymorphic protein from swine whey.

tyrosine to the extent of about 5–10 per cent. Methionine and cystine were determined independently as methionine sulphone and cysteic acid, respectively, after performic acid oxidation according to the method of Moore (1963). The absence of cysteine was confirmed by the use of Ellman's reagent (1959). Cystine was also determined by use of Ellman's reagent after reduction with $NaBH_4$ by the method of Heinselman, Phillips, and Jenness (1968). The tryptophan content of each polymorph was determined spectrophotometrically by using the ultra-violet absorptivities of Beavan and Holiday (1952) and Wetlaufer (1962) for cystine, tyrosine, and tryptophan and the known content of cystine and tyrosine. The ultra-violet spectra of the proteins were obtained in a water solution at *p*H 6·0 in a Cary Recording Spectrophotometer Model 14. In

addition, tryptophan was determined after protective alkaline hydrolysis and pronase pretreatment of the proteins according to the method of Spies (1967). The amide nitrogen was calculated after performic acid oxidation according to Stegemann (1958).

Isoionic Point

The isoionic point was determined using a mixed bed ion-exchange resin as described by Dintzis (1952).

Terminal Amino-acids

The Edman degradation as applied for paper strips by Fraenkel-Conrat, Harris, and Levy (1955) and Schroeder, Shelton, and Shelton (1961) was employed for the qualitative determination of the *N*-terminal amino-acids of the swine whey proteins. Chromatography with Sjøquist solvent A (Fraenkel-Conrat and others (1955), and visualization with the starch–iodide detection method proved satisfactory for the identification of the phenylthiohydantoin (PTH) amino-acids released.

The *N*-terminal group was verified by using the (1-fluoro-2,4-dinitrobenzene) (FDNB) method of Sanger as outlined by Fraenkel-Conrat and others (1955).

For the determination of the C-terminal amino-acids of the native proteins (M.W. ~18,000) carboxypeptidase A was employed. The enzyme was obtained from Worthington Biochemical Corporation and was treated with di-isopropyl-fluorophosphate prior to use. The reaction and quantitation of the amino-acids released were carried out as previously described by Kalan, Greenberg, and Walter (1965) for bovine β-lactoglobulins, except that the reaction was terminated after 48 hours.

Comparison of Tryptic Digests of Swine Proteins and Bovine β-Lactoglobulin B

Approximately 6·5 μ*M* (~120 mg.) of each of the 3 proteins (M.W. ~18,000) were performic acid oxidized by the method of Stegemann (1958) to disrupt disulphide bonds. Digestions with trypsin (weight ratio of enzyme to substrate 1:120) were carried out under nitrogen in 10-ml. volumes using unbuffered systems and maintaining *p*H at 8·0 by automatic addition of 0·1 *N* NaOH in a *p*H-stat. The reaction was carried out for 4 hours at 37° C. with the addition of a second aliquot of trypsin after 2 hours to bring the final enzyme to substrate ratio to 1:60. The reaction was terminated by freezing the solution, which was then lyophilized. The digests were analysed by high-voltage electrophoresis on Whatman 3 MM paper, using a horizontal water-cooled plate, and the buffers and techniques of Ingram (1958). Single-dimension electrophoresis was carried out at *p*H 6·4 for 2 hours with a potential of 40 V. per cm. The digests were applied as streaks (1·0 mg. per 0·5 in.) and the peptide patterns visualized as described by Kalan, Gordon, Basch, and Townend (1962). Two-dimensional chromatograms were obtained by applying 2·0 mg. of each digest, as a spot on a single paper and following the procedure outlined by Kalan, Greenberg, and Thompson (1966).

RESULTS AND DISCUSSION

Isolation of Proteins

Kraeling and Gerrits (1967) first observed the possible existence of a polymorphic whey protein in sow's milk by the use of gel electrophoresis in veronal buffer, *p*H 8·6. *Fig.* 2 is a record of an electrophoretic run of skim milk of individual sows. The fastest moving component represents the proteins of interest. It is noted that the BB protein is somewhat diffuse compared to the faster moving AA protein. A survey by Kraeling and Gerrits (1967) of individual sows with 3 breeds of different background indicated from segregation analysis and the Hardy-Weinberg equilibrium formula that the polymorphism is controlled by 2 codominant autosomal alleles. That is, the heterozygous condition results in the appearance of both proteins in the milk. This is indicated in *Fig.* 2. Kraeling and Gerrits (1969) have now confirmed and extended their original findings. The figure also reveals relatively few discrete protein bands, and there is a streaking out of protein material from the origin reminiscent of non-reduced bovine κ-casein behaviour on gels, first observed by Wake and Baldwin (1961). The number of bands increases markedly when urea is added to the electrophoresis medium. In this connexion it has been observed that the difference in mobility between the polymorphs disappears in the presence of urea and dimethylformamide, and in the presence of β-mercaptoethanol in the absence of dissociating reagents. It was also found that the difference in mobility becomes evident only above *p*H 6·0. As will be noted below, the only compositional difference between the proteins found from analysis appears to reside in the non-polar amino-acids, alanine and valine. Hence, it is presumed that this difference in electrophoretic mobility results from

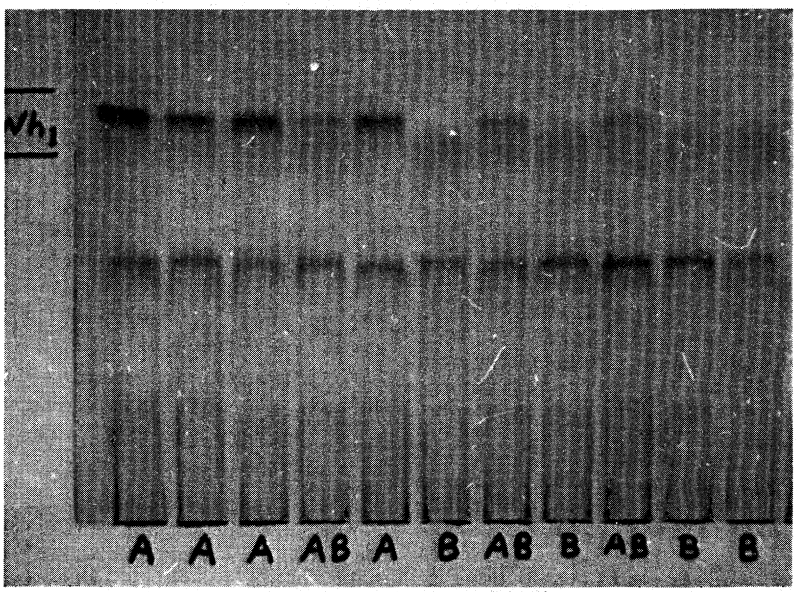

FIG. 2.—Gel electrophoresis of skim milk of individual cows; 5 per cent polyacrylamide gel, *p*H 8·6 veronal buffer, 0·025 ionic strength.

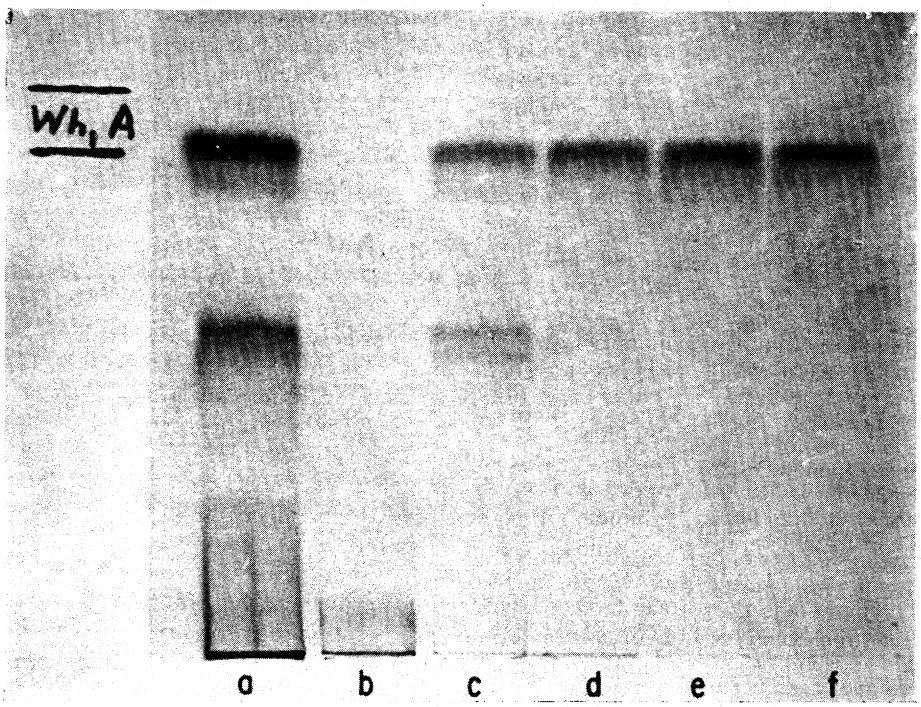

FIG. 3.—Gel electrophoresis of some fractions obtained during preparation of crystalline whey protein. a, Skim milk. b, Casein. c, 20 per cent Na_2SO_4, *p*H 6·6. d, 20 per cent Na_2SO_4, *p*H 3·0. e, 2·3 *M* $(NH_4)_2SO_4$. f, 1 × recrystallized protein.

dealing with a globular protein precipitating at *p*H 6·0 with 2·3 *M* $(NH_4)_2SO_4$. The protein is isolated in a similar fashion to that of bovine α-lactalbumin in the method of Aschaffenburg and Drewry (1957b). A further point of interest is the essential absence of a fraction in swine whey corresponding to the β-lactoglobulin fraction of cow whey found by the method of Aschaffenburg and Drewry (1957b). The protein did not substitute for either the A or B protein in the lactose synthetase system of Brodbeck and Ebner (1966) (Ebner, 1969). Ebner, Denton, and Brodbeck (1966), Ebner and Brodbeck (1966), and Brodbeck, Denton, Tanahashi, and Ebner (1967) have shown that the B protein of lactose synthetase is identical to cow α-lactalbumin, and that swine whey has a protein which can substitute for the B protein. In this connexion it is interesting to note that swine whey has no antigen which will react with rabbit anti-serum to cow

Table I.—Amino-acid Composition of Crystalline Swine Whey Polymorphs*

Amino-acid	Swine AA		Swine BB		Cow β-Lactoglobulin B‡ (residues)	Cow α-Lactalbumin§ (residues)
	Average†	Residues	Average†	Residues		
Asp	17·32	17	17·19	17	15	22
Thr	9·76 ‖	10	9·75 ‖	10	8	7
Ser	9·72 ‖	10	9·93 ‖	10	7	7
Glu	22·96	23	22·76	23	25	14
Pro	8·07	8	8·21	8	8	2
Gly	3·06	3	3·18	3	4	7
Ala	13·05	13	14·02	14	15	4
½-Cys	3·77 ‖	4	3·81 ‖	4	4	8
	4·11 ¶		4·13 ¶			
			3·40 **			
Val	12·90	13	11·74	12	9	6
Met	3·78	4	3·65	4	4	1
	3·93 ¶		3·72 ¶			
Ile	5·92	6	5·90	6	10	8
Leu	24·16	24	23·88	24	22	14
Tyr	1·89	2	1·90	2	4	5
Phe	3·00	3	3·00	3	4	4
Lys	10·72	11	10·94	11	15	12
His	2·98	3	3·01	3	2	3
Arg	5·01	5	4·81	5	3	1
Trp	1·14 ††	1	1·20 ††	1	2	5
	0·89 ‡‡		0·94 ‡‡			
	0·96 ‖‖		0·96 ‖‖			
Cys	< 0·005	0	< 0·005	0	1	0
Amide N	9·88	10	9·72	10	14	15
Total residues		160		160	162	130
Molecular wt.		17,860		17,832	18,278	15,544

* Results are expressed as residues per mole of protein (M.W. ~18,000).
† *See* text for determination of averages.
‡ Data taken from Piez and others (1961), Gordon and others (1961), and Kalan and others (1965).
§ Data taken from Gordon and Ziegler (1955) in essential agreement with Brew and others (1967).
‖ Calculated for linear regression to zero time.
¶ Calculated as cysteic acid and methionine sulphone respectively after performic acid oxidation.
** Calculated as cysteine after $NaBH_4$ reduction.
†† Calculated by ultra-violet absorption.
‡‡ Calculated after protective alkaline hydrolysis.
‖‖ Calculated after pronase treatment.

α-lactalbumin (Lyster and others, 1966). Hence, though the swine protein has certain properties similar to α-lactalbumin, such as isolation behaviour, solubility, and the absence of free sulphydryl groups (*see below*), it is apparently not homologous to cow α-lactalbumin.

(Nirenberg, Leder, Bernfield, Brimacombs, Turpin, Rottman, and O'Neal, 1965). No carbohydrate was detected by use of the Schiff reagent as applied to gels (Purkayastha and Rose, 1965).

A comparison of the composition of the swine proteins with the two most abundant

Table II.—Comparison of Swine Proteins and Cow β-Lactoglobulins by Grouping of Amino-acids

Amino-acids	Swine AA	Swine BB	β-Lg A	β-Lg B	β-Lg C
Asp Asn Glu Gln	40	40	41	40	39
Ser Thr	20	20	15	15	15
Pro	8	8	8	8	8
Gly Ala Val Ile Leu	59	59	59	60	60
Met ½-Cys Cys	8	8	9	9	9
Tyr Phe Trp	6	6	10	10	10
Lys His Arg	19	19	20	20	21
Amide N	10	10	14	14	13

Amino-acid Composition

The amino-acid composition of the swine whey proteins is given in *Table I* together with literature values for the composition of cow β-lactoglobulin B and α-lactalbumin for purposes of comparison. It can be seen that no calculated value is more than 0·3 of a residue different from its whole number residue value, except for Asp (AA) and Met (BB). The low ½-Cys value calculated as cysteine after $NaBH_4$ reduction cannot be explained. Further, a comparison of the average values obtained for each of the proteins reveals that no amino-acid in the AA protein differs from the corresponding amino-acid in the BB protein by more than 0·28 of a residue except for Ala and Val. Swine AA has one residue more of Val and one residue less of Ala than the BB protein. Thus the only compositional difference between the polymorphs resides in the difference in content of a pair of non-polar amino-acids. A similar amino-acid difference was found in cow β-lactoglobulin A and B (Gordon, Basch, and Kalan, 1961; Piez, Davie, Folk, and Gladner, 1961) and can be coded by the nucleotide triplets GG $\begin{matrix}U\\C\\A\\G\end{matrix}$/GU $\begin{matrix}U\\C\\A\\G\end{matrix}$ for Ala/Val

bovine whey proteins indicates a closer relationship of the swine protein to bovine β-lactoglobulin. Of the invariant amino-acids Pro, ½-Cys, and Met are identical for the swine protein and cow β-lactoglobulins A, B, and C (Kalan and others, 1965). For the variant amino-acids, β-lactoglobulin A has 3 Gly and 14 Ala and β-lactoglobulin C has 3 His, all values identical to one or the other of the swine polymorphs. Further, the molecular weight and total residues are virtually identical for the swine proteins and cow β-lactoglobulins, but very different from cow β-lactalbumin. The only similarity in composition of the swine polymorphs to α-lactalbumin appears to be the absence of cysteine. The similarity between cow β-lactoglobulins and the swine proteins is made more evident by the grouping of amino-acids shown in *Table II*. It is seen that the greatest differences reside in content of hydroxy amino-acids, aromatic amino-acids, amide nitrogen, and the presence of cysteine in the β-lactoglobulins. The greater content of aromatic amino-acids in β-lactoglobulin accounts for the higher specific extinction coefficient for this protein. Nozaki, Bunville, and Tanford (1959) determined a value of 9·4, and using the known content of Tyr, Trp, and Cys, and the molar extinctions

given by Wetlaufer (1962), a value of 9·2 can be calculated. The swine proteins have spectra identical in shape to that of the β-lactoglobulins shown by Townend, Herskovits, Swaisgood, and Timasheff (1964), with a maximum between 275 and 285 mμ, and a shoulder at 290 mμ. A specific extinction coefficient of 5·2–5·4 was calculated from the ultra-violet absorption, resulting in the Trp values shown in *Table I.* This value is somewhat higher than the values obtained by chemical analysis (Spies, 1967). If the specific extinction coefficient is calculated on the basis of amino-acid composition and the molar absorptivities given by Wetlaufer (1962), a value of 4·8 is obtained for the swine proteins. The value of ~1·1 for the ratio of the observed to calculated specific extinction coefficients is in agreement with the value for this ratio obtained with other proteins (Wetlaufer, 1962).

Table III compares the number of potential anionic and cationic groups in the swine proteins and β-lactoglobulin B. It is seen that the swine polymorphs have an excess of 11 potential anionic groups and an isoionic point of 4·78–4·80 compared to an excess of 6 potential anionic groups and an isoionic point of 5·35 for β-lactoglobulin B (Basch and Timasheff, 1967). This difference of 0·55–0·57 *p*H unit resulting from a difference of 5 potential anionic groups could be expected on the basis of the work of Basch and Timasheff (1967) and Tanford and Nozaki (1959) with the β-lactoglobulin variants. Finally, the fact that both swine polymorphs have the same number of potential cationic and anionic groups would seem to rule out charge differences as being responsible for the difference in mobility in alkaline gel. This, however, is a tentative conclusion until titration studies with the swine polymorphs are carried out.

In the report of Kessler and Brew (1970), no indication of polymorphism was observed, and this is attributed to the fact that all milk samples were derived from a single inbred herd of pigs. The protein these workers isolated appears to be the AA polymorph containing equal numbers of residues of valine and alanine, that is, 13. Kraeling and Gerrits (1969) have shown that in the Duroc breed they observed no homozygous BB milks and only 9 AB heterozygous milks in a total of 92 milks screened. It would appear that the reason given by Kessler and Brew for not observing polymorphism is valid. An alternative explanation may be the electrophoretic method employed for screening. The present authors used vertical slab gels in contrast to disk-gels used by Kessler and Brew. Nevertheless, both groups of investigators agree in end-group analysis, sulphydryl content, specific extinction coefficient, molecular weight,

Table III.—Comparison of Potential Cationic and Anionic Groups in Swine Proteins and Cow β-Lactoglobulin B

Group	Swine Proteins	Cow β-Lactoglobulin B
Potential cationic		
ε-NH_2	11	15
Guanido	5	3
Imidazole	3	2
N-Terminal-NH_2	1	1
Total	20	21
Potential anionic		
β, γ-COOH	40	40
C-Terminal-COOH	1	1
-β, γ-$CONH_2$	10	14
Total	31	27
Excess potential anionic	11	6
Isoionic point	4·78–4·80	5·35

and immunological tests. There are some differences in amino-acid content for serine (10 residues as against 11 found by Kessler and Brew), glutamic acid (23 as against 24), lysine (11 as against 13), and arginine (5 as against 6). This results in a molecule of 160 amino-acids calculated to a molecular weight of 17,860 for the AA polymorph as opposed to a molecule of 165 residues and molecular weight of 18,517 for the protein of Kessler and Brew. It is apparent, however, that both groups are involved with the same protein and have reached identical and independent conclusions concerning its relationship to ruminant β-lactoglobulin.

identical for each variant. A full equivalent of Val is released within 2 hours, but subsequent amino-acids are hydrolysed slowly, reminiscent of the behaviour of cow β-lactoglobulins with carboxypeptidase A after the initial rapid hydrolysis of the *C*-terminal-His–Ile sequence (Kalan and others, 1965). The penultimate amino-acid in the swine proteins appears to be Leu, although a full equivalent is not released up to 48 hours of incubation. An analogy can be made with the slow release of Leu, the antepenultimate amino-acid in cow β-lactoglobulins (Kalan and others, 1965). It is thus seen that the swine proteins have the identical *N*- and

Table IV.—Comparison of Several Ruminant β-Lactoglobulins, Cow α-Lactalbumin, and Swine Proteins with Respect to Terminal Amino-acids

Whey Protein	*C*-Terminal Amino-acids	*N*-Terminal Amino-acids
Cow α-lactalbumin*	-Lys-Leu	Glu-
Cow β-Lactoglobulins A, B, C†	-Leu-His-Ile	Leu-Thr-
Goat β-Lactoglobulin‡	-His-Val	Ile-
Sheep β-Lactoglobulin	-His-Val§	Phe-‖
Swine whey protein¶	-Leu-Val	Val-

* From Weil and Seibles (1961).
† From Kalan and others (1965).
‡ From Phillips and Jenness (1965).
§ From Maubois and others (1965).
‖ From Kalan (1968).
¶ Present paper.

Terminal Amino-acids

Chromatographic identification of the *N*-terminal amino-acid released by the Edman degradation revealed Val as the end-group. Further sequence could not be elucidated as the chromatograms became too complex for interpretation, although Lys or Glu appeared to be the amino-acid following Val. The fact that Val was the *N*-terminal amino-acid was confirmed by the use of the Sanger reagent, FDNB. DNP-Val was the only ether soluble DNP-amino-acid found, confirming the results of the Edman procedure. The results were identical for each swine polymorph.

The results of the incubation of the swine proteins with carboxypeptidase A were also

C-terminal amino-acids, Val, and the Ala/Val difference must occur within the polypeptide chain.

Table IV is a comparison of several β-lactoglobulins, cow α-lactalbumin, and the swine proteins with respect to the *N*- and *C*-terminal amino-acids. It can be seen that all β-lactoglobulins, as well as the swine protein, have hydrophobic N-terminal amino-acids. α-Lactalbumin has an acidic glutamic acid *N*-terminal. Similarly all β-lactoglobulins have a hydrophobic *C*-terminal amino-acid with penultimate His. The swine protein has *C*-terminal Val with penultimate Leu, identical to the antepenultimate residue found in cow β-lactoglobulins. α-Lactalbumin has a *C*-terminal

Leu followed by Lys. From these data it is possible to draw a sequence of possible mutations in the triplet genetic code (Nirenberg and others, 1965) to account for the different terminal amino-acids in the β-lactoglobulins and the swine protein. For the *C*-terminal Ile/Val change, the triplet change would be $\underset{\text{AUC/GUC;}}{\overset{\text{U}\quad\text{U}}{}}$ for the *N*-terminal Leu/Phe/Ile/Val changes, the triplet changes would be $\underset{\text{CUC/UUC/AUC/GUC.}}{\overset{\text{U}\quad\text{U}\quad\text{U}\quad\text{U}}{}}$ The absence of penultimate His in the swine protein could be due to a deletion mutation, with Leu, the antepenultimate amino-acid in cow β-lactoglobulins, appearing as the penultimate amino-acid in the swine protein.

a case for homology could not be made. However, it should be pointed out that Brew, Vanaman, and Hill (1967) have demonstrated a possible homology between hens' egg-white lysozyme and cow α-lactalbumin, prompted by the observation of Brew and Campbell (1967) that the proteins have similar molecular weights, identical or similar numbers of some amino-acids, the same number of disulphide bonds, and similar or identical *N*- and *C*-terminal residues. The similarity of these properties can be invoked in the case of β-lactoglobulins and the polymorphic swine proteins of the present report. Finally, it can be reported that no peptide differences could be detected on a comparison of the two-dimensional chromatograms of the

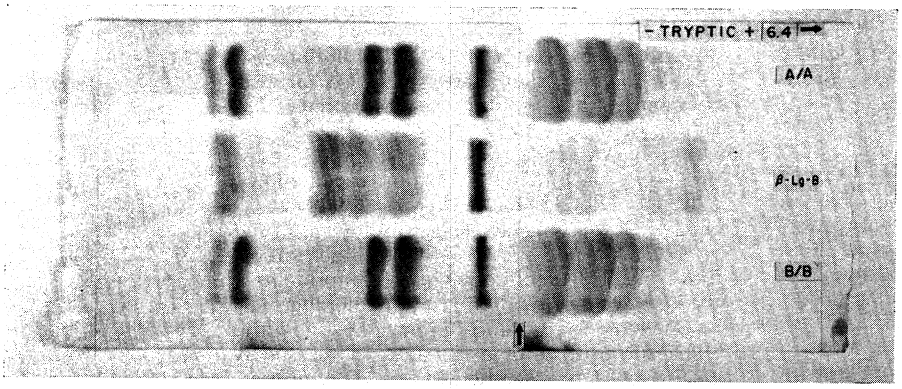

FIG. 5.—Single-dimension high-voltage electrophoretogram of tryptic digests of performic acid oxidized swine whey protein (AA and BB) and cow β-lactoglobulin B (β-Lg B), 40 V. per cm. for 2 hours at *p*H 6·4.

COMPARISON OF TRYPTIC DIGESTS OF SWINE PROTEINS AND BOVINE β-LACTOGLOBULIN B

A further test of the possible homology of the swine polymorphs with β-lactoglobulin was the comparison of peptide patterns after tryptic digestion of the performic acid oxidized proteins. *Fig.* 5 is a single-dimension high-voltage electrophoretogram of such a proteolytic digestion. The 2 polymorphs show identical peptide patterns which, however, appear to be quite different from that arising from cow β-lactoglobulin B. Two dimensional patterns also revealed complete identity between the swine polymorphs and many differences between the swine and the cow proteins. On this basis, it would appear that swine proteins. This result is not unexpected on the basis of the non-polar Ala/Val amino-acid difference between the polymorphs.

CONCLUSION

A polymorphic crystalline protein has been isolated from swine whey by salt and *p*H fractionation. The protein crystallizes from solutions containing 52–58 per cent $(NH_4)_2SO_4$. The only difference in composition between the polymorphs is in Ala and Val content. The difference in content of these amino-acids apparently gives rise to a difference of electrophoretic mobility in alkaline gels. This difference disappears in the presence of dissociating reagents, disulphide-

reducing agents, and reduced *p*H, indicating that differences in conformation and/or ion binding resulting from compositional differences are responsible for the electrophoretic mobility difference of the polymorphs. The polymorphs are not homologues of cow α-lactalbumin on the basis of failure to cross-react immunologically and to replace α-lactalbumin in the lactose synthetase enzyme system. In addition, compositional and molecular weight differences appear to rule out such an homology. Despite the failure of the swine proteins to cross-react with antisera to cow β-lactoglobulin and the obvious differences of peptide maps of tryptic digests of cow and swine proteins, a case can be made for homology of swine protein and β-lactoglobulins on the basis of similarity of molecular weights, identical or similar numbers of some amino-acids, same number of disulphide bonds, and similar or identical *N*- and *C*-terminal residues.

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Key Word Index: Non-ruminant β-lactoglobulin, swine whey protein polymorphism, isolation, crystallization and partial characterization of a swine polymorphic protein.